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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)					
Given Name (first and middle [if any])		Family Name or Surname		Residence (City and either State or Foreign Country)	
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Additional inventors are being named on the <u>separately numbered sheets attached hereto</u>					
TITLE OF THE INVENTION (500 characters max)					
Materials and Methods for Control of Pests					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number		23557			
OR					
<input type="checkbox"/> Firm or <input type="checkbox"/> Individual Name					
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ENCLOSED APPLICATION PARTS (check all that apply)					
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<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE	
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees.				AMOUNT (\$)	
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
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Respectfully submitted

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Date June 29, 2004

REGISTRATION NO. 46,853

(if appropriate)

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No. : UF-428P  
Applicants : Russell F. Mizell III, Peter C. Anderson  
For : Materials and Methods for Control of Pests

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## DESCRIPTION

### MATERIALS AND METHODS FOR CONTROL OF PESTS

5

#### Background of the Invention

*Homalodisca coagulata* (Say), the glassy-winged sharpshooter (GWSS), perhaps represents the single greatest threat to the agroecology of California in the history of the state. GWSS is capable of establishment throughout the grape growing regions of CA (Hoddle 2004). As a result, the threat to grape production from Pierce's Disease, *Xylella fastidiosa* Wells et al., has increased dramatically. The long-term impact on CA of this invasive vector is unknown, but will likely be more severe than the relatively short term economic impact to date. Currently, ca. \$133 million in producer, state and federal funds have been expended in CA in efforts to research, suppress, control and contain this pest. Citrus, grape and nursery industries have been severely impacted by the statewide effort to contain GWSS. Other crops such as almonds, peaches and plums are also at risk.

The first line of defense against GWSS is to eliminate the spread of the vector to uncolonized areas of the state through regulatory efforts and to prevent establishment of new populations through early detection and eradication. During 2002, approximately 2500 infested or adjacent properties were treated in six CA counties during rapid response activities and over 65,000 loads of commodities were inspected for GWSS (Anonymous 2003). Presently, the primary tools available for regulatory suppression and eradication are early detection followed by chemical pesticide applications (Redak and Blua 2003). The CDFA web site containing the GWSS nursery shipping protocol lists the following chemicals with efficacy against GWSS: acephate, cyfluthrin, methiocarb, bifenthrin, deltamethrin, permethrin, fenpropathrin, carbaryl, chlorpyrifos and imidaclopid. Many of these chemicals have logistical limitations including long reentry intervals and other potential side effects that restrict their use or result in added environmental costs as well as elicit severe negative reactions from the public. The mode of action of all of the currently-recommended chemicals registered for use against GWSS is by direct or indirect (feeding suppression by

neonicotinoids, repellency by kaolin clay, Surround) mortality to the targeted life stages. One of the biggest problems in efforts to contain the spread of GWSS is the lack of effective treatments for GWSS egg masses that occur on many different host plants. Eliminating or limiting the occurrence of egg masses with presently available tools, may now be possible, yet these avenues have not been fully researched.

Redak and Bethke (2003) summarized the results of the previous evaluations of pesticides against the GWSS. A large number of chemicals have been evaluated against GWSS life stages that include commercially-available organic, biorational and reduced-risk chemicals. Evaluations of the efficacy of the chemicals were based primarily on mortality to the target stages. Moreover, the results from most previous evaluations were based on short-term tests using typical laboratory and field protocols whereby the mortalities of untreated control organisms are compared to treated individuals over a period of hours or days. Some insect growth regulators, primarily synthetic chitin inhibitors, have been tested over a period of several weeks and found to be effective against GWSS nymphs but caused no adult mortality. However, Redak and Bethke (2003) concluded that the activity of these compounds (buprofizen, novaluron and pyriproxifen) was too slow to be useful for eradication purposes. Other researchers (Akey et al. 2003) have evaluated certain biorationals including cinnamon oil, pyrethrum and piperonyl butoxide - for use in organic production and found limited efficacy against GWSS.

#### Brief Summary of the Invention

To our knowledge, no previous efforts have not been made to evaluate the efficacy of currently labeled juvenile hormone analogs: methoprene, kinoprene and hydroprene. These materials may have direct and indirect impacts on the behavior, reproductive or other physiological systems of GWSS. Moreover, the potential long-term impact of treatments to nymphs on the subsequent reproductive activities of adult GWSS has not been evaluated. In Appendix I, we provide some preliminary results indicating that at least one of these compounds, methoprene, eliminates GWSS oviposition by irreversibly suppressing the development of the female reproductive system. We also have data indicating this chemical has no impact on the egg parasitoids, *Gonatocerus* spp. and is therefore a biorational

chemical tool that can be readily integrated with other chemical and biological controls. Moreover, this class of chemicals will readily satisfy demands of the environmentally-sensitive public.

The term insect growth regulator (IGR) loosely covers a variety synthetic analogs that mimic hormones and related chemicals essential for chitin synthesis (Staal 1975). Our interest in this project is with the juvenile hormone analogs listed above that we will refer to as JH analogs. JH is produced in the endocrine system of insects and can mediate or impact a broad array of physiological functions including ecdysis, metamorphosis, diapause, reproduction and metabolism. Six major members of the juvenile hormone group are currently recognized and affect processes in both male and female arthropods (Klowden 2002). Some affects are the disruption of embryogenesis and ecdysis as well as acute effects on reproductive development including male and female sterilization. Additionally, JH analogs can also cause indirect mortality through the impairment of sensory functions, behavior, feeding, mating, etc. JH analogs are efficacious at extremely low concentrations in micro to nanogram amounts (Staal 1975). In many insect species JH analogs disrupt diapause either by terminating diapause out of season or by making diapause permanent, therefore causing sterilization. In some insects, treatment of males with JH analogs has produced negative effects on the reproduction of the females who mate with the treated male (Stall 1975). Relatively little biochemical rationale is available for predicting the impact of JH analogs on individual species or life stages of arthropods. The mode of action of JH analogs varies greatly between the members of Insecta. As a result, intensive experimentation with individual species and each chemical must be conducted to determine impacts.

Some previous work with IGRs on leafhoppers has been reported. Juvenile hormone III terminated diapause in the leafhopper *Draeculacephala crassicornis* by topical, substrate and vapor treatments. Other compounds affected nymph metamorphosis and female reproductive diapause (Kamm and Swenson 1972, Reissig and Kamm 1973). In a related planthopper, *Peregrinus maidis* (Heteroptera: Delphacidae) one of the most sensitive morphological structures proved to be the female ovipositor. Treated females had shortened ovipositors as the only visible sign of inhibition and produced eggs that were only half

inserted into plant tissue. These eggs did not hatch successfully (Staal 1975). In Appendix I we present evidence that methoprene has severe impact on GWSS female reproduction. For most JH analogs, direct toxic effects are generally absent and all effects are indirect. This necessitates thorough experimentation to determine the potential of these compounds. This is most likely the reason that heretofore these compounds have not been investigated against GWSS and related vectors. This is surprising given the available literature cited above, the biorational characteristics of the compounds, and the fact that they are currently being used extensively in CA (Appendix II) against several groups of insect pests in a myriad of indoor (Diacon II – fleas, Gentrol – food areas) and outdoor target sites including aquatic (Altosid - mosquitoes) and most importantly CA nursery production (Enstar II - Heteroptera).

Methoprene, isopropyl (2E, 4E)- 11-methoxy-3, 7, 11 trimethyl-2,4-dodecadienoate, is registered by EPA as Precor for flea control, Extinguish Fire Ant Bait, Altosid for larval mosquito control and Diacon II for stored product insect pests in cereal grains, sweet corn, popcorn, birdseed and peanuts. Methoprene does not kill adult insects directly but often adversely affects the immature stages. It is labeled both for indoor and outdoor use, requires no protective equipment to use and has no reentry interval. Kinoprene, [2-propynyl (2E, 4E)-(7S)-3,7,11-trimethyl-2, 4-dodecadienoate], is labeled as Enstar II IGR for control of whiteflies, aphids, soft and armored scales, mealybugs, and fungus gnats in greenhouses and interiorscapes on ornamental plants. Kinoprene has a 12 hour reentry interval. Hydroprene, ethyl (2E, 4E)-3,7,11-trimethyl-2,4-dodecadienoate, is labeled as Gentrol and Turbocide Gold for use in stored products and for cockroach control in both food and non food areas. These compounds can be tank mixed with other products.

IGRs, specifically these labeled synthetic juvenile hormone analogs, offer a unique mode of action with little, if any, nontarget side effects. They offer an alternative treatment that is highly compatible with and complementary to other IPM tools and programs. The chemicals are already available and are being used in nursery production for other pests where GWSS is presently causing great economic impact. As stated above, the biggest problem in nursery production relative to regulatory needs is the production and placement of GWSS eggs. This problem requires great expenditure of time and effort to detect the eggs and represents a high risk pathway for the spread of GWSS to uncolonized areas. Our



preliminary data suggest that we can suppress or eliminate GWSS oviposition by treated females. We have discovered (data Appendix 1, overview below) that methoprene effectively sterilizes GWSS females. This chemical class (to our knowledge) has not been previously evaluated for use against GWSS or the impacts were not detected due to the tedious methods  
5 required for effective evaluations. Understanding the complete impacts and timing of how this compound and other related compounds disrupt GWSS physiology and behavior are essential to the development of effective field control strategies that can be used in conjunction with already established IPM strategies.

We topically applied this compound in an aqueous solution to GWSS females  
10 (Appendix I) that were: 1) overwintering in reproductive diapause or 2) females that were newly eclosed adults and not yet reproductively active. Diapausing females that were treated remained reproductively inactive for at least 30 days after they were placed into summer conditions (32°C, 14:10 L:D photoperiod) that caused the untreated control females to begin ovipositing after 10 days. Additionally, newly eclosed females did not develop eggs or  
15 oviposit after treatment with this chemical, even after 36 days of summer conditions. Untreated females ordinarily will begin reproductive cycles 10-12 days post eclosion. Treated reproductively-active females oviposited the eggs they contained then stopped for the following weeks of evaluation.

Treating GWSS nymphs of stage with this material did not cause mortality, increase or  
20 decrease time of development, or induce morphological aberrations. We have yet to determine if treating nymphs affects subsequent adult oviposition. It is also possible that other untested formulations, rates or analogs of this chemical may directly affect nymphs.

We quantified the efficacy of this material only after targeted dissection of the reproductive system of adult females. In our investigations, we have developed a baseline  
25 understanding of the normal sequence of events that occur during reproduction, including fat body deposition and utilization, brochosome development, maturation of ova, and oviposition. This candidate material inhibits the development of both brochosomes and ovarioles. Treated females have a high amount of accumulated fat body but do not produce eggs for at least 36 days post treatment. Thirty-six days is the maximum amount of time so

far tested, but greatly exceeds the 10-12 days after adult eclosion that normal GWSS females require after to begin oviposition.

In conclusion, our database on the GWSS female reproductive cycle suggests that environmental conditions encountered by nymphs greatly impact subsequent oviposition as adults. Potentially, nymphal exposure to this compound may also impact adult fecundity. These effects would not be noted in the initial discovery screenings that were performed previously by researchers. With this exciting preliminary data and knowledge of the previous research on the myriad effects of JH analogs on an array of insects species, we propose to evaluate the effects of the labeled compounds for their impact on the physiology and behavior of GWSS, its parasites, *Gonatocerus* spp, and for comparison purposes two related leafhopper species, *Homalodisca insolita* and *Oncometopia nigricans*. We will conduct tests with the two related vectors to get an idea of how broad the impacts of the IGRs may be on this group of insects so that extrapolation can be made to other vector species in and outside CA.

#### Detailed Description of the Invention

We will screen the compounds methoprene, kinoprene and hydroprene to further elucidate their potential efficacy against GWSS and related vectors. We will evaluate a range of rates of the compounds against all GWSS life stages and those of *Oncometopia nigricans* and *Homalodisca insolita* to determine LD<sub>50</sub>s. The tests will require holding the insects for >21 days post treatment on host plants of good quality for observations of feeding, mating, oviposition and other behaviors. Oviposition rates will be quantified and each female dissected to evaluate reproductive status.

We will systematically evaluate the life stages (eggs, 1-2 instars, 4-5 instars, adults) of the three leafhopper species, as well as target the mating and oviposition behavior of diapausing and actively breeding adult males and females. We will develop and evaluate treatments that assess the residual and potential reversal of activity on female oviposition. We will also evaluate the compounds for their impact on the GWSS egg parasites, *G. ashmeadi* and *G. morrelli* by treating GWSS eggs containing the parasite larvae and by treating the adult parasites. The experiments will be conducted in the laboratory and

greenhouse using field and greenhouse-raised leafhoppers. For each treatment 10-20 individuals will be used in each of 3-5 replicates. Treatments will be first applied directly to the insects using an aerosol spray apparatus. Serial dilutions for each compound tested will be evaluated to establish LD<sub>50</sub>s. Post treatment the insects will be held on appropriate host plants in the greenhouse and observed for mortality and indirect impacts on their behavior and physiology such as lack of molting, prolonged stadium length, mating and oviposition status. Treatments of adult females will require dissection of the reproductive system and these will be described and quantified as in the preliminary data in Appendix I. We will: 1) determine the effects on molting and longevity of each chemical on each life stage of the 3 leafhopper species and the parasites, 2) determine the impact of each chemical on the behavior and reproductive systems of male and female leafhoppers, 3) establish the dose response relationships of any observed impacts using serial dilutions, and 4) provide a report including recommendations to CDFA as to how to use the new tools against GWSS

We have a well-documented commitment to solving the GWSS/PD problem and have produced some of the benchmark publications on GWSS feeding behavior and nutrition as well as many other aspects of GWSS/PD biology and interactions. Our preliminary data and long track record of publications indicates that we are readily capable of achieving the proposed objectives. The NFREC-Quincy just moved into a new \$6 mil laboratory with all inside and outdoor accoutrements to conduct, demonstrate and transfer the latest cutting-edge scientific research and technology in most agricultural disciplines in relation to all commodities grown in Zones 7-9. The Center is well equipped with the latest in equipment: tractors, seeders, cultivators, harvesters, irrigation equipment, greenhouses, dryers, storage facilities, etc. A farm manager and labor crew support faculty field and laboratory research and all the PIs have full or part-time technical support. Annual research and demonstration includes all aspects of the culture and management practices required to grow and recommend production practices for vegetables, small grains, fruit crops, forage crops, nursery and landscape management and other minor and exotic crops. In addition, personnel at the Center conduct basic and applied research projects on IPM (all pest types), water and soil science, livestock production and forage, and plant breeding (wheat, small grains, peanut, soybean).

The present state of knowledge on PD/GWSS suggests that solving the problem will require a long-term research effort focused on a variety of objectives aimed at the biology, behavior and interactions between the bacterium, the vector and the host plants. If a “silver bullet” is discovered it will likely be something that directly affects the bacterium. However, what areas will be most fruitful or the time frame necessary to develop and implement a primary solution is not predictable. At this time, during perhaps a transition of 5-10 years or longer, we must continue to address the problem from as many promising perspectives as possible including chemical, cultural and management methods for controlling the spread and establishment of the vector. There is no other way to preserve the multi-billion dollar industries that are affected by this potentially devastating problem during the transition period. This modest proposal offers a new approach that will impact the vector in several novel ways not currently being investigated by addressing egg deposition in nursery crops and the establishment of new colonies. Moreover, the tools are readily available, safe and environmentally benign. What remains is to develop the background data on what, when and how they affect GWSS physiology and behavior so that the tools can be used most effectively.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

## **Appendix I: Preliminary Results and Materials and Methods Used.**

**Example 1: Females in diapause.** Ten female *Homalodisca coagulata* (Say) were sprayed until visibly wet with the candidate compound. They were then placed into a wooden 1m screened cage that was provisioned with five males and glabrous soybean, *Glycine max* (L.) A similar untreated control cage was also set up with females sprayed with distilled water. All leafhoppers were taken from a greenhouse culture and were in the process of terminating

winter reproductive diapause. Females were checked daily for the presence of brochosomes and plants were checked for egg masses. Cages were in a greenhouse maintained at 32°C and equipped with artificial lighting for a 14:10 photoperiod. Surviving females were dissected after thirty days and their reproductive status was evaluated.

- 5        Results. No eggs were produced by any treated GWSS females. Dissections revealed that all surviving treated females had not begun reproductive activity, even after 30 days (Table. 1). There was little or no brochosome material in the Malpighian tubules and no development of ova. All control females were reproductively active.

10    **Table 1. Status of reproductive structures of *H. coagulata* females treated with compound.**

Treated				
Female #	Ovarioles	Fat body	Brochosomes	Ova
1	2	2	1	1
2	2	2	1	1
3	2	2	1	1
Untreated				
Female #	Ovarioles	Fat body	Brochosomes	Ova
1	3	1	2.5	2
2	3	2	3	2
3	3	1.5	3	2
4	3	1.5	3	2
5	3	1.5	3	2
6	3	1.5	3	3

15    Key:

Ovarioles	Ova
1=small, little development	1=none
2=fully developed; no ova	2=single developing ova per ovariole
3=fully developed with ova	3=two or more developing ova per ovariole

20

Fat body	Brochosomes (within Malpighian tubules)
1=minimal	1=very little; tubule is translucent
2=medium	2=medium; tubule is opaque white, not swollen
3=heavy	3=heavy; tubule is opaque white, swollen

25

#### **Example 2: Newly-eclosed adult females**

Seven newly eclosed adult female *H. coagulata* were treated with the compound. The solution was applied with an aerosol device until individuals were noticeable wet. After

treatment, female were placed into a wooden 1m-screened cage that was provisioned with crape myrtle, eastern saltbush, and soybean. Five males of unknown ages were added to the cage. Female were dissected after 36 days. Males were discarded.

Results. No eggs were produced by any treated GWSS females (Table 2.). Dissection of treated females indicated that ovariole (reproductive) development was inhibited. Under normal green house conditions, female *H. coagulata* will begin to oviposit 10-12 days after eclosion. A few days prior to oviposition, they have swollen bodies and begin to display brochosomes on the forewings.

10 **Table 2. Reproductive status of females 36d post-treatment.**

Female #	Ovarioles	Fat body	Brochosomes	Ova
1	2	1.5	1.5	1
2	2	2	1	1
3	2	2	1	1
4	2	2	1	1
5	2	2	1	1
6	2	2.5	1	1
7	2	1.5	1	1

**Appendix II. Use of juvenile hormone analogs in CA in 2002**  
([www.pesticideinfo.org/detail\\_chemUse.jsp?Rec\\_ID=PC32837](http://www.pesticideinfo.org/detail_chemUse.jsp?Rec_ID=PC32837)).

**Top 50 Crops and Sites for for Methoprene use in California in 2002** Top ↑

<u>Crop or Site</u> (Commodity Code)	<u>Gross Pounds</u>	<u>Application Rate</u> pounds per acre treated	<u>Acres Planted</u> where all or part has been sprayed	<u>Acres Treated</u>	<u>Application Count</u>
<u>All Sites (00)</u>	5,121	-	-	-	2,128
<u>Public Health Pest Control (50)</u>	4,942	-	-	-	844
<u>Structural Pest Control (10)</u>	157.3	-	-	-	1,252
<u>Landscape (30)</u>	13.3	-	-	-	23
<u>Right of Way (40)</u>	8.64	-	-	-	6
<u>Regulatory (100)</u>	0.02	-	-	-	3

**Regional****Top 50 Crops and Sites for (S)-Kinoprene use in California in 2002**

Top ↑

<u>Crop or Site</u> (Commodity Code)	<u>Gross Pounds</u>	<u>Application Rate</u> pounds per acre treated	<u>Acres Planted</u> where all or part has been sprayed	<u>Acres Treated</u>	<u>Application Count</u>
<u>All Sites</u> (00)	192.2	0.29	1,681	633.3	321
<u>Greenhouse Flowers</u> (151)	80.7	0.33	194.5	241.0	83
<u>Greenhouse Plants</u> (153)	54.4	0.29	124.3	189.2	85
<u>Outdoor Container Nursery</u> (154)	46.3	0.26	1,314	177.4	81
<u>Landscape</u> (30)	3.18	-	-	-	16
<u>Commodity Research</u> (99)	2.92	-	-	-	25
<u>Outdoor Flower Nursery</u> (152)	2.74	0.25	19.5	10.8	16
<u>Greenhouse Propagation</u> (155)	1.95	0.13	29.0	15.0	12
<u>Structural Pest Control</u> (10)	0.03	-	-	-	3

**Top 50 Crops and Sites for Hydroprene use in California in 2002**

Top ↑

<u>Crop or Site</u> (Commodity Code)	<u>Gross Pounds</u>	<u>Application Rate</u> pounds per acre treated	<u>Acres Planted</u> where all or part has been sprayed	<u>Acres Treated</u>	<u>Application Count</u>
<u>All Sites</u> (00)	1,656	-	-	-	4,039
<u>Structural Pest Control</u> (10)	1,633	-	-	-	3,961
<u>Landscape</u> (30)	15.7	-	-	-	62
<u>Other Fumigation</u> (91)	3.52	-	-	-	6
<u>Commodity Research</u> (99)	1.87	-	-	-	1
<u>Regulatory</u> (100)	1.67	-	-	-	2
<u>Vertebrate Pest Control</u> (80)	0.26	-	-	-	2
<u>Commodity Fumigation</u> (90)	0.16	-	-	-	1
<u>Right of Way</u> (40)	0.10	-	-	-	4

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Abstract

The present invention pertains to compounds and methods for controlling pests, such as the glassy-winged sharpshooter (GWSS), and their parasites. In one embodiment, the compound is a juvenile hormone analog such as methoprene, kinoprene, and hydropene. The invention also concerns pesticidal compositions comprising the compounds of the invention and a pesticidally acceptable carrier. The invention further concerns methods for controlling pests, such as GWSS, and/or their parasites, by applying a compound or composition of the invention to the pest or pest-inhabited locus.